

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problems Mailbox.**

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: A61L 27/00, A61K 35/38	A1	(11) International Publication Number: WO 98/19719 (43) International Publication Date: 14 May 1998 (14.05.98)
(21) International Application Number: PCT/US97/20240 (22) International Filing Date: 4 November 1997 (04.11.97) (30) Priority Data: 60/030,211 5 November 1996 (05.11.96) US (71) Applicant (for all designated States except US): PURDUE RESEARCH FOUNDATION [US/US]; Room 307, 1021 Hovde Hall, West Lafayette, IN 47907-1021 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): GEDDES, Leslie, A. [US/US]; 400 North River Road #1724, West Lafayette, IN 47906 (US). BADYLAK, Stephen, F. [US/US]; 1150 Kingswood Drive #S, West Lafayette, IN 47906 (US). MATHENY, Robert, G. [US/US]; 12746 Devon Lane, Carmel, IN 46032 (US). SCHOENLEIN, William, E. [US/US]; 6304 South 450 East, Lafayette, IN 47905 (US). OBERMILLER, Fred, J. [US/US]; Young Grad. House #818, West Lafayette, IN 47907 (US). HAVEL, William, J. [US/US]; 5034 Wolflake Drive, Lafayette, IN 47905 (US). (74) Agent: LAMMERT, Steven, R.; Barnes & Thornburg, 11 South Meridian Street, Indianapolis, IN 46204 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: MYOCARDIAL GRAFT CONSTRUCTS		
(57) Abstract The use of submucosal tissue of a warm blooded vertebrate to manufacture a tissue graft composition that induces the formation of endogenous cardiac tissues <i>in vivo</i> upon contact of the cardiac tissues with the manufactured composition.		

Serial No. 09/706,307
Docket No. 441742001200

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon		Republic of Korea	PT	Portugal		
CN	China	KR	Republic of Korea	RO	Romania		
CU	Cuba	KZ	Kazakhstan	RU	Russian Federation		
CZ	Czech Republic	LC	Saint Lucia	SD	Sudan		
DE	Germany	LJ	Liechtenstein	SE	Sweden		
DK	Denmark	LK	Sri Lanka	SG	Singapore		
EE	Estonia	LR	Liberia				

-1-

MYOCARDIAL GRAFT CONSTRUCTSField of the Invention

The present invention relates to a tissue graft
5 composition and methods for its preparation and use. More
particularly, the present invention is directed to non-
immunogenic submucosal tissue graft compositions prepared
from warm-blooded vertebrates and the use of those
compositions to promote growth of endogenous cardiac
10 tissues.

Background and Summary of the Invention

It is known that compositions comprising the
tunica submucosa of the intestine of warm-blooded
15 vertebrates can be used as tissue graft materials. See
U.S. Patent Nos. 4,902,508 and 5,281,422. The tissue graft
compositions described in those patents are characterized
by excellent mechanical properties, including a high burst
pressure, and an effective porosity index which allows such
20 compositions to be used beneficially for vascular graft and
connective tissue graft constructs. When used in such
applications the graft constructs appear not only to serve
as a matrix for the regrowth of the tissues replaced by the
graft constructs, but also promote or induce such regrowth
25 of endogenous tissue. Common events to this remodeling
process include: widespread and very rapid
neovascularization, proliferation of granulation
mesenchymal cells, biodegradation/resorption of implanted
intestinal submucosal tissue material, and absence of
30 immune rejection.

It is also known that intestinal submucosa can be
fluidized by comminuting and/or enzymatic digestion,
without loss of its apparent biotrophic properties, for use
in less invasive methods of administration (e.g., by

-2-

injection or topical application) to host tissues in need of repair. See U.S. Patent No. 5,275,826.

Surprisingly, it has been found that submucosal tissue is also capable of promoting endogenous regrowth and healing of damaged or diseased cardiac tissues, including the endocardium, pericardium, and myocardium. Myocardial tissue comprises the middle muscular tissue of the heart wall. Cardiac muscle tissue differs from smooth and skeletal muscle in that the nuclei are centrally located, the cells form a syncytium, and the cells exhibit the property of spontaneous contractibility or "automaticity."

In accordance with the present invention submucosal tissue of a warm blooded vertebrate is used for the preparation of a graft construct that promotes endogenous regrowth and healing of damaged or diseased cardiac tissues upon contact of said damaged or diseased tissues with the composition.

The present submucosal tissue graft compositions can be implanted or injected into a vertebrate host to induce the repair or replacement of damaged or defective heart tissues. In one embodiment damaged or diseased myocardial tissues are contacted *in vivo* with a composition comprising intestinal tunica submucosa of a warm blooded vertebrate to enhance the formation of endogenous tissues having spontaneous contractile properties.

Brief Description of the Drawings

Fig. 1 illustrates myographic data for remodeled canine ventricular myocardium. Spontaneous contractions (1, 2 and 3) and an evoked contraction (50 ms, 160 mA) were observed in the remodeled tissue from the MV2 dog.

Fig. 2 is a graphic representation of strength-duration data for remodeled canine ventricular myocardium of the MV2 dog (Rheobase = 55.42 mA).

-3-

Fig. 3 illustrates myographic data for remodeled canine atrial septum in the MA1 dog when the tissue was exposed to electrical stimulation (50 ms, 160 mA).

Fig. 4 is a graphic representation of strength-duration data for remodeled canine atrial septal tissues of the MA2 dog (Rheobase = 30.9 mA).

Fig. 5 is a graphic representation of strength-duration data for remodeled canine ventricular myocardium of the MV1 dog (Rheobase = 139 mA).

Fig. 6 is graphic representation of strength-duration data for normal canine ventricular tissue of the MV1 dog (Rheobase = 55.42 mA).

Detailed Description of the Invention

There is provided in accordance with this invention a method and composition for promoting the repair of damaged or diseased heart tissues. Generally the method comprises the step of contacting target cells *in vivo* with a vertebrate derived collagenous matrix. The collagenous matrix compositions of the present invention can be injected or implanted into a host to induce the formation of endogenous heart tissues including the formation of spontaneously contractile myocardial tissues.

The collagenous matrix for use in preparing the compositions of the present invention can be selected from a variety of commercially available collagen matrices or can be prepared from a wide variety of natural sources of collagen. In preferred embodiments the collagenous matrix for use in accordance with the present invention comprises highly conserved collagens, glycoproteins, proteoglycans, and glycosaminoglycans in their natural configuration and natural concentration. Most preferably the collagenous matrix comprises submucosa-derived tissue of a warm-blooded vertebrate. Submucosal tissue can be obtained from various vertebrate organ sources (such as intestinal tissue)

-4-

harvested from animals raised for meat production, including, for example, pigs, cattle and sheep or other warm-blooded vertebrates.

The submucosal tissue used in accordance with the present invention is preferably derived from the intestines, more preferably the small intestine, of a warm blooded vertebrate. Preferred intestinal submucosal tissue typically comprises the tunica submucosa delaminated from both the tunica muscularis and at least the luminal portions of the tunica mucosa. In one preferred embodiment of this invention the submucosal tissue comprises the tunica submucosa and basilar portions of the tunica mucosa including the lamina muscularis mucosa and the stratum compactum. The preparation of submucosal tissue for use in accordance with this invention is described in U.S. Patent No. 4,902,508, the disclosure of which is expressly incorporated herein by reference. A segment of vertebrate intestine, preferably harvested from porcine, ovine or bovine species, is first subjected to gentle abrasion using a longitudinal wiping motion to remove both the outer layers, identified as the tunica serosa and the tunica muscularis, and the innermost layer, i.e., the luminal portions of the tunica mucosa. The submucosal tissue is rinsed with water or saline, optionally sterilized, and can be stored in a hydrated or dehydrated state. Delamination of the tunica submucosa from both the tunica muscularis and at least the luminal portions of the tunica mucosa and rinsing of the submucosa provides an acellular matrix designated as submucosal tissue. The use and manipulation of such material for the formation of ligament and tendon grafts and the use more generally of such submucosal tissue constructs for inducing growth of endogenous connective tissues is described and claimed in U.S. Patent No. 5,281,422 issued January 25, 1994, the disclosure of which is expressly incorporated herein by reference.

-5-

It is also known that fluidized forms of submucosal tissue can be prepared without loss of the submucosal tissue's ability to induce the growth of endogenous tissues. Fluidized submucosa compositions
5 comprise comminuted submucosa or enzymatically treated submucosa, and in one embodiment the submucosal tissue is comminuted and enzymatically treated to form a substantially uniform or homogenous solution. In one
embodiment, the submucosa is treated with a protease, such
10 as trypsin or pepsin, or other appropriate enzymes for a period of time sufficient to solubilize said tissue and form a substantially homogeneous solution. The preparation of fluidized forms of intestinal submucosa is described in U.S. Patent No. 5,275,826, the disclosure of which is
15 expressly incorporated herein by reference.

The present invention also contemplates the use of powder forms of submucosal tissues. In one embodiment a powder form of submucosal tissue is prepared by pulverizing intestinal submucosa tissue under liquid nitrogen to
20 produce particles ranging in size from 0.01 to 1 mm in their largest dimension. The particulate composition is then lyophilized overnight, pulverized again and optionally sterilized to form a substantially anhydrous particulate composite. Alternatively, a powder form of submucosal
25 tissue can be formed from fluidized submucosal tissue by drying the suspensions or solutions of comminuted submucosal tissue. Both solid and fluidized forms of intestinal submucosa have been found, upon implantation or injection to induce endogenous remodeling processes
30 including rapid neovascularization, proliferation of granulation mesenchymal cells, resorption of the implanted submucosa tissue and absence of immune rejection. *In vivo*, implanted submucosa tissue has been found effective to induce the proliferation and growth of cells/tissues with
35 which it is in contact or which it replaces.

-6-

Because of the unique characteristics and highly specialized nature of myocardial tissue and the knowledge that damaged myocardial tissue heals by scar (non-contractile) tissue formation in mammals, it was not anticipated that submucosal tissue could induce the formation of endogenous myocardial tissues. Surprisingly, the submucosa graft constructs of the present invention promote endogenous regrowth and healing of damaged or diseased cardiac tissues (including the endocardium, pericardium, and myocardium) when the graft constructs are placed in contact with the endogenous cardiac tissues and exposed to the same natural microenvironment as the endogenous cardiac tissues.

Submucosal tissues used in accordance with the present invention lend themselves to a wide variety of surgical applications relating to the repair or replacement of damaged heart tissues, including, for example the repair or replacement of myocardial, endocardial and pericardial tissues. Submucosal tissue grafts are used to induce the formation of endogenous myocardial tissue at a desired site in the heart of a warm blooded vertebrate. The submucosal tissue compositions can be administered to the host in either solid sheet, strip or loop form, by surgical implantation, or in fluidized form, by injection. Solid sheet and strip forms of submucosal tissue have been previously described in US application no. 08/418,515 and PCT application serial no. PCT/US96/04721 the disclosures of which are expressly incorporated herein by reference. The loop form of submucosal tissue comprises a continuous strip of submucosal tissue formed into a multilaminate structure by wrapping the strip onto itself and around at least one axis. Typically the ends of the strip of submucosal tissue forming the loop are immobilized by suturing, gluing, stapling, compressing or utilizing other

-7-

tissue immobilizing techniques known to those skilled in the art to fix the ends to the remaining graft construct.

One embodiment of the present invention comprises a method for enhancing the formation of spontaneously contractile myocardial tissues. The method comprises contacting heart myocardial tissues *in vivo* with a composition comprising intestinal submucosal tissue in an amount effective to promote endogenous cardiac tissue growth at the site the composition is administered.

10 In accordance with one embodiment of the present invention the ability of submucosal tissue to induce the formation of excitation-propagating myocardial tissue is utilized to create an A-V bridge to propagate excitation from the atria to the ventricles in a subject having A-V
15 block. In the United States about 100 thousand cardiac pacemakers are implanted annually. A large majority of these are used to pace the ventricles because the A-V conductive system has been blocked.

Heart block is a disorder of the heart beat that
20 leads to episodes of dizziness or fainting. Heart block is caused by an interruption to the passage of impulses through the specialized conducting system of the heart. Consequently, although the atria beat normally, the ventricles lag behind or contract less often than the
25 atria. In complete heart block the atria and ventricles beat independently; thus while the rate of atrial contractions varies according to the patients activity, the ventricles contract at a fairly constant rate of about 40 beats per minute. In a large percentage of these patients
30 the natural pacemaker (S-A node) and the atria function normally. In these patients submucosal tissue can be implanted into the atrial and ventricular myocardium to remodel into myocardial tissue capable of conducting excitation from the S-A node. Thus, the atrial excitation
35 will be propagated along the submucosal remodeled A-V

-8-

bridge and the atria will drive the ventricles, thereby eliminating the need for an implanted cardiac pacemaker in patients having a normally function S-A node and atria but suffering from A-V block.

5 The surgical procedure of implanting the submucosal tissue into the atrial and ventricular myocardium may be performed with the aid of a laparoscope, thereby requiring minimal invasive surgery. The implanted submucosal tissue remodels to atrial and ventricular
10 myocardium capable of conducting excitation from the S-A node to the ventricles. Complete remodeling of the implanted submucosal tissue takes approximately four to eight weeks, during which time a temporary external pacemaker may be used if needed. Accordingly, the use of a
15 submucosal tissue implant will allow the ventricles to be driven by the atria such that the rate of contraction will automatically increase with exercise. Furthermore, no implantable pacemaker is needed and the repair of the A-V bridge should last the entire life of the patient.

20 In another embodiment fluidized submucosal tissue can be injected into or adjacent to a site in need of endogenous cardiac tissue growth to induce the formation of cardiac tissues such as the myocardium, pericardium and endocardium. For example, submucosal tissue can be
25 injected between the pericardium and the myocardium in an amount effective to induce repair of the damaged or diseased tissue. Alternatively the fluidized submucosal tissue can be injected directly into the damaged or diseased heart tissue in need of repair.

30 In an alternative embodiment damaged or diseased portions of the heart can be repaired by surgically replacing the affected tissue with a patch of submucosal tissue in solid sheet, strip or loop form. Consistent with the use of submucosal tissue as a patch graft material for
35 replacement of damaged or diseased myocardial tissues,

-9-

preferred vertebrate intestinal submucosa tissue possesses mechanical properties highly desirable for such tissue graft materials, including low porosity index, a high burst pressure, and a stratum compactum surface with low
5 thrombogenicity.

The submucosa grafts formed and used in accordance with this invention, upon implantation, serve as a rapidly vascularized matrix for support and growth of new endogenous cardiac tissue. The graft material is remodeled
10 (resorbed and replaced with autogenous differentiated tissue) and assumes the characterizing features of the heart tissue with which it is associated at the site of implantation. Indeed, where a single graft "sees" multiple microenvironments as implanted, it is differentially
15 remodeled along its length and thickness. Thus, for example, when used in myocardial tissue replacement studies the graft appears to develop endocardial tissue as well as myocardial tissues.

For myocardial replacement applications
20 submucosal tissue graft constructs are typically "conditioned" to alter the viscoelastic properties of the submucosal tissue. Submucosal tissue is conditioned by stretching, chemically treating, enzymatically treating or exposing the tissue to other environmental factors. The
25 conditioning of submucosal tissue is described in U.S. Patent No. 5,275,826, the disclosure of which is expressly incorporated herein by reference. In accordance with one embodiment vertebrate derived submucosal tissues are conditioned to a strain of no more than about 20%.

30 In one embodiment the submucosal tissue is conditioned by stretching the graft material longitudinally to a length longer than the length of the submucosal tissue from which the graft construct was formed. One method of "conditioning" the tissue by stretching involves
35 application of a given load to the submucosa for three to

-10-

five cycles. Each cycle consists of applying a load to the graft material for five seconds, followed by a ten second relaxation phase. Three to five cycles produces a stretch-conditioned graft material. The graft material does not
5 immediately return to its original size; it remains in a "stretched" dimension. For example, submucosal tissue can be conditioned by suspending a weight from the tissue, for a period of time sufficient to allow about 10 to 20% or more elongation of the tissue segment. Optionally, the
10 graft material can be preconditioned by stretching in the lateral dimension. The graft material exhibits similar viscoelastic properties in the longitudinal and lateral dimensions.

In one embodiment the submucosal tissue is
15 stretched using 50% of the predicted ultimate load. The "ultimate load" is the maximum load that can be applied to the submucosal tissue without resulting in failure of the tissue (i.e. the break point of the tissue). Ultimate load can be predicted for a given strip of submucosal tissue
20 based on the source and thickness of the material. Accordingly, one method of "conditioning" the tissue by stretching involves application of 50% of the predicted ultimate load to the submucosa for three to ten cycles. Each cycle consists of applying a load to the graft
25 material for five seconds, followed by a ten second relaxation phase. The resulting conditioned submucosal tissue has a strain of less than 30%, more typically a strain from about 20% to about 28%. In one preferred embodiment conditioned the submucosal tissue has a strain
30 of no more than 20%. The term strain as used herein refers to the maximum amount of tissue elongation before failure of the tissue, when the tissue is stretched under an applied load. Strain is expressed as a percentage of the length of the tissue before loading.

-11-

The conditioned submucosal tissue can be packaged and stored to maintain the conditioned state of the tissue. In one embodiment in accordance with the present invention, the conditioned submucosa is part of an article of
5 manufacture that comprises submucosa and a support, wherein the support maintains the submucosa in a conditioned state. The conditioned submucosa and support can be enclosed within a container and sterilized to maintain the sterility of the submucosa. Alternatively, the conditioned submucosa
10 and the support can be sterilized before enclosing the material within the container, and optionally exposed to a second round of sterilization after sealing the container. In one embodiment the conditioned submucosa is intestinal submucosa, and more particularly, comprises the tunica
15 submucosa delaminated from both the tunica muscularis and at least the luminal portions of the tunica mucosa.

Accordingly, a package for storing graft material ready for use in a surgical procedure comprises, sterile submucosal tissue of a warm-blooded vertebrate, a support,
20 and a sealed container. In this embodiment, the submucosal tissue is conditioned by stretching the submucosal tissue from a first length to a second length that is longer than the first length, the support maintains the tissue at its stretched second length, and the sealed container encloses
25 and maintains the sterility of the submucosal tissue and the support. In one embodiment the submucosal tissue is stretched/conditioned to have a strain of less than 30%, and in another embodiment the tissue is stretched/conditioned to have a strain of no more than 20%.

30 The submucosal tissue can be conditioned before it is wrapped onto a spool, block or frame or it can be conditioned while, or as, the tissue is wrapped onto the spool, block or frame. In one embodiment, the submucosal tissue is conditioned while it is wrapped onto the spool,
35 block or frame by fixing the end of a strip/sheet of

-12-

conditioned submucosal tissue onto the spool, block or frame and wrapping the submucosal tissue onto the spool, block or frame under tension. The second end of the submucosal tissue is then immobilized to maintain the preconditioned length of the wrapped submucosal tissue.

For example, intestinal submucosa that has been delaminated from the tunica muscularis and at least the luminal portions of the tunica mucosa can be fed between a series of paired rollers, the free end attached to a spool (or other rotatable object) and rolled onto the spool. In one embodiment at least two sets of the paired rollers rotate at different speeds relative to one another, to place tension on the tissue located between the two rollers. The intestinal submucosa is thus stretched to a length greater than the original length of the delaminated intestinal submucosa and is immediately wrapped onto the spool to maintain the stretched condition of the intestinal submucosa. By varying the relative speed of the paired rollers the intestinal submucosa can be wrapped onto the roller and have a preselected strain.

Alternatively the conditioned intestinal submucosa can be held on a rigid frame to maintain the submucosa in its conditioned state. In one embodiment, the frame has opposite sides that are held in fixed position relative to one another, and the opposite sides are each provided with immobilizing means for immobilizing said submucosal tissue. The immobilizing means can be selected from clamps, sutures, staples, pins, glues or other tissue immobilizing techniques known to those skilled in the art. Preferably the frame is adjustable to several locked positions that vary the distance between the opposite sides to allow for various lengths of submucosa to be held within the frame and under tension.

Intestinal submucosa exhibits similar viscoelastic properties in all directions, i.e. the

-13-

material can be stretched in any dimension to reduce the strain of the material. The material does not immediately return to its original size; it remains in a "stretched" condition. Accordingly, the delaminated intestinal

5 submucosa can be stretched in a first dimension along a first axis and then stretched in a second dimension along a second axis wherein the first and second axis are not parallel to one another. In one embodiment the delaminated submucosa is stretched in a first dimension along a first

10 axis and then stretched in a second dimension along a second axis, wherein the first axis and second axis are perpendicular to one another, to produce a graft construct that has a width and length longer than the original delaminated tissue.

15 Typically the conditioned submucosal tissue is immobilized by clamping, suturing, stapling, gluing (or other tissue immobilizing techniques) the tissue to the support, wherein the tissue is held at its preconditioned length in at least one dimension. In one embodiment the

20 delaminated intestinal submucosa is conditioned to have a width and length longer than the original delaminated tissue and the conditioned length and width of the tissue is maintained by immobilizing the submucosa on a support. The support-held conditioned submucosal tissue can be

25 sterilized before or after being packaged.

Unitary large area sheets of submucosal tissue and multi-layered constructs, as described in US application no. 08/418,515 and PCT application serial no. PCT/US96/04721 the disclosures of which are expressly

30 incorporated herein by reference, can be utilized in accordance with the present invention to form graft constructs for heart tissue repair. These multi-laminate constructs are formed by overlapping individual strips of submucosal tissue and applying pressure to the overlapped

35 portions to fuse the tissues together. In one embodiment

-14-

pressure is applied to the overlapped tissue under conditions allowing dehydration of the submucosal tissue.

The present submucosal tissue constructs may be sterilized using conventional disinfection/sterilization techniques including tanning with glutaraldehyde, formaldehyde tanning at acidic pH, ethylene oxide treatment, propylene oxide treatment, gas plasma sterilization, gamma radiation or electron beam treatment, and peracetic acid disinfection. Sterilization techniques which do not significantly weaken the mechanical strength and biotropic properties of the graft are preferred. For instance, strong gamma radiation may cause loss of strength of the sheets of submucosal tissue. Preferred sterilization techniques include exposing the graft to peracetic acid, 1-4 Mrads gamma irradiation (more preferably 1-2.5 Mrads of gamma irradiation) or gas plasma sterilization. Typically, the submucosal tissue is subjected to two or more sterilization processes. After the submucosal tissue is treated in an initial disinfection step, for example by treatment with peracetic acid, the tissue may be wrapped in a plastic or foil wrap and sterilized again using electron beam or gamma irradiation sterilization techniques.

25 Examples

Repair of Atrial Septal and Ventricular Defects

Intestinal submucosal tissue grafts were implanted in four dog hearts. Isoflurane anesthesia and cardiopulmonary bypass were used during the procedure. In two of the four dogs an atrial septal defect was made, about the size of a nickel, and the defect was patched with peracetic acid cleansed porcine intestinal submucosa delaminated from both the tunica muscularis and at least

-15-

the luminal portions of the tunica mucosa. These dogs were designated MA1 and MA2.

In the remaining two dogs (MV1 and MV2), a hole the size of a quarter, was made in the right ventricle near the base, and then patched with small intestinal submucosal tissue delaminated from both the tunica muscularis and at least the luminal portions of the tunica mucosa. The submucosal tissue was implanted with the luminal side facing the blood. The luminal surface of the intestinal submucosal tissue is the surface facing the lumen of the organ source and typically adjacent to an inner mucosa layer *in vivo* whereas the abluminal surface is the submucosal surface facing away from the lumen of the organ and typically in contact with smooth muscle tissue *in vivo*. The luminal surface is smoother and generally has more thrombo resistance than the abluminal surface.

Approximately eleven weeks after implantation, MA1 and MV2 were barbiturate-anesthetized, positive-pressure ventilated and the chests were opened. The hearts were then photographed *in situ*. The location of the submucosal tissue graft patch on the right ventricle of MV2 was visible as a suture ring about one half of the diameter of the size when the patch was made originally. The pericardium was adherent to the graft site. The ventricles were fibrillated electrically and a 1 x 1 cm specimen was removed from the center of the graft area. A 1 x 1 cm specimen was also excised from adjacent ventricular native myocardium and compared to the remodeled graft. The remodeled graft tissue was found to be slightly thinner than adjacent native ventricular myocardium.

The excised specimen of the remodeled MV2 graft was tested for its ability to contract using the following technique. Sutures were applied to the four corners of the graft. With this arrangement the contractile force could be measured along three axes. The remodeled graft tissue

-16-

was mounted in an oxygenated tissue bath containing Krebs solution maintained at 37°C. and connected to a myograph.

The MV2 specimen remodeled graft tissue exhibited spontaneous contractions and relaxations at an irregular
5 slow-rate, as shown in Fig. 1 (1,2,3). In addition the tissue was tested to determine if the tissue was responsive to electrical stimuli. A single stimulus was delivered to plate electrodes in the tissue bath. These electrodes were not in contact with the specimen, and thus the stimulating
10 current was carried by the tissue-bath fluid. The remodeled graft tissue (MV2) contracted in response to a single stimulus, as shown in Fig. 1 (50 ms, 160 mA). A strength-duration curve was obtained, i.e. a plot of the strength of a threshold stimulus needed to evoke a
15 contraction versus stimulus duration. Fig. 2 shows the strength-duration curve for the remodeled graft tissue of MV2, exhibiting a chronaxie of 1.08 ms; the chronaxie for normal myocardium is typically 1.4 ms. A strength-duration curve was not obtained for the adjacent native myocardium.

20 As noted above, the MA1 dog, implanted with the atrial septal graft, was barbiturate-anesthetized, positive-pressure ventilated, the chest opened and the heart was photographed. The ventricles were fibrillated electrically and the heart was removed. The atrial septal
25 graft was removed and examined. The atrial septal graft was firmer than the surrounding myocardium and slightly more pale. No blood clots (thrombi) were found in the right ventricle or in either atrium.

Sutures were placed in the corners of the
30 remodeled atrial septal graft. When mounted in the tissue bath, no spontaneous contractions were evident. Response to single electrical stimuli was recorded as shown in Fig. 3. It is not surprising that the contraction was weak because septal tissue is not known to be contractile.

-17-

Table 1 summarizes the dates when the implants were made and when those specimens were explanted.

5

TABLE 1 - IMPLANT & EXPLANT DATES

	ANIMAL	IMPLANT	EXPLANT	DAYS
	MA1	1/22/96	4/9/96	78
	MA2	1/23/96	6/18/96	148
10	MV1	1/24/96	6/18/96	147
	MV2	1/24/96	4/9/96	78

Histological Examination of Excised Tissues

15 Histologic examination of the remodeled right
ventricular free wall graft (MV2) showed bundles of
cardiomyocytes surrounded by organized collagenous
connective tissue. The cardiomyocytes were morphologically
normal with observable striations, centrally located
20 nuclei, and a syncytial arrangement. Approximately 50% of
the remodelled tissue was myocardial cells and the
remaining 50% was collagenous connective tissue. The
boundary between the graft and the normal ventricular wall
was barely apparent (confirmed by suture placement) and
25 there was bridging of this anastomotic site by the
cardiomyocytes. Masson's trichrome stain confirmed the
presence of these cardiomyocytes with an eosinophilic
staining spindle cell containing striations and central
nuclei. The tissue was highly vascularized. There was a
30 normal endocardial lining.

 Histologic examination of the remodeled
interatrial septum graft showed a mixture of connective
tissues including cardiomyocytes surrounded by a fibrous
(collagenous) connective tissue and islands of cartilage,
35 and small clusters of adipocytes. The cardiomyocytes were
morphologically normal but occupied a small (perhaps 10 to
15%) of the total area. The fibrous connective tissue
occupied the majority of the area which was examined.
Cartilage appeared to represent approximately 20% of the

-18-

area which was examined. The blood contacting surfaces of the interatrial graft site showed morphologically normal endocardium.

As shown in Table 1, MA2 survived for 148 days and MV1 for 147 days before sacrifice. MV1 experienced no complications during the study. However, MA2 developed atrial fibrillation during the 17 weeks after implantation.

Histologic examination of the remodeled atrial septal defect graft tissue of MA2 showed a mixture of connective tissue types within the tissue which once consisted of submucosal tissue. The mixture of tissues included myocardial tissue, well differentiated cartilage, fibrous connective tissue, and adipose connective tissue. The atrial lining consisted of endothelial cells. There was no evidence for an inflammatory reaction.

Histopathologic examination of the tissues taken from the right ventricular free wall graft site of MV1 showed the endocardial surface contained an intact endothelial layer with a deep accumulation of bundles of developing and well differentiated myocardial cells. These bundles of cells were of variable size and orientation and were associated with a small amount of fibrous connective tissue. The epicardial connective tissue replacement consisted of a thick bank of dense fibrous connective tissue. All tissues were highly vascularized. There was no evidence of any inflammatory cells in any of the tissues or sections examined. The myocardial tissue comprised approximately 65% of the graft area in the dog which survived 147 days.

30

Physiological Studies MA2 and MV1.

The remodeled atrial and ventricular grafts were placed in the tissue bath and connected to a myograph. No spontaneous contractions and relaxations were observed in either the remodeled atrial or ventricular grafts.

35

-19-

However, both were responsive to electrical stimulation and strength-duration curves were obtained, as shown in Fig. 4 (MA2) and Fig. 5 (MV1). A strength-duration curve was obtained for normal MV1 canine ventricle (Fig. 6). Table 2 summarizes the chronaxie values for all 4 dogs.

TABLE 2 CHRONAXIE VALUES

10	ANIMAL NUMBER	CHRONAXIE REMODELED	(MSEC) NATIVE
	MA1	-----	-----
	MA2	1.35	-----
15	MV1	1.32	1.63
	MV2	1.08	-----

-20-

Claims:

1. The use of submucosal tissue of a warm blooded vertebrate for the preparation of a graft construct
5 that promotes endogenous regrowth and healing of damaged or diseased cardiac tissues.

2. The use of submucosal tissue in accordance with claim 1 wherein the submucosal tissue comprises
intestinal submucosa delaminated from both the tunica
10 muscularis and at least the luminal portion of the tunica mucosa.

3. The use of submucosal tissue in accordance with claim 1 wherein the submucosal tissue has a strain of less than 30%.

15 4. The use of submucosal tissue in accordance with claim 1 wherein the submucosal tissue has a strain of no more than 20%.

5. A method for promoting endogenous regrowth and healing of damaged or diseased cardiac tissues, said
20 method comprising contacting said damaged or diseased tissues with a composition comprising submucosal tissue of a warm blooded vertebrate.

6. The method of claim 5 wherein the step of contacting the damaged or diseased cardiac tissues
25 comprises surgically implanting a patch of submucosal tissue at the site in need of repair.

7. The method of claim 6 wherein the submucosal tissue is conditioned so that the tissue has a strain of less than 30%

30 8. The method of claim 6 wherein the submucosal tissue is conditioned so that the tissue has a strain of no more than 20%.

9. The method of claim 5 wherein the submucosal tissue is fluidized and the fluidized tissue is injected

-21-

into or adjacent to said damaged or diseased cardiac tissues.

10. A method for repairing damaged or diseased portions of a warm blooded vertebrate heart, said method
5 comprising replacing the damaged or diseased tissue with a graft composition comprising submucosal tissue of a warm blooded vertebrate.

11. The method of claim 10 wherein the submucosal tissue is implanted into the atrial and
10 ventricular myocardium to remodel into myocardial tissue that functions as an A-V bridge for conducting excitation from the S-A node to the ventricles.

12. A method for enhancing the formation of spontaneously contractile myocardial tissues, said method
15 comprising contacting heart myocardial tissues with a composition comprising intestinal submucosal tissue in an amount effective to promote endogenous tissue growth at the site the composition is administered.

13. The method of claim 12 wherein the
20 submucosal tissue comprises intestinal submucosa delaminated from both the abluminal muscle layers and at least the luminal portion of the tunica mucosa of a warm-blooded vertebrate.

14. The method of claim 13, wherein the graft
25 composition is administered by surgically implanting the composition into the warm-blooded vertebrate.

15. The method of claim 14 wherein the submucosal tissue is conditioned so that the tissue has a strain of less than 30%.

30 16. The method of claim 15, wherein the graft composition is fluidized and is administered by injection into the warm-blooded vertebrate.

17. An article of manufacture comprising intestinal submucosa and a support wherein the intestinal
35 submucosa is conditioned to have a strain of less than 30%

-22-

and the support holds and maintains the tissue in its conditioned state.

18. The article of manufacture of claim 17 further comprising a sealed container that encloses and
5 maintains the sterility of said intestinal submucosa and said support.

19. The article of manufacture of claim 17 wherein the intestinal submucosa comprises the tunica submucosa delaminated from both the tunica muscularis and
10 at least the luminal portions of the tunica mucosa.

20. The article of manufacture of claim 17 wherein the support is a spool and the intestinal submucosa is wrapped onto the spool under sufficient tension to maintain the length of the conditioned submucosal tissue.

15 21. The article of manufacture of claim 17 wherein the support is a frame having opposite sides for holding said intestinal submucosa, wherein said opposite sides are held in fixed position relative to one another.

22. The article of manufacture of claim 21
20 wherein at least one of the opposite sides further comprises a clamp.

23. A package for storing graft material ready for use in a surgical procedure, said package comprising sterile submucosal tissue of a warm-blooded
25 vertebrate that has been conditioned by stretching from a first length to a second length longer than said first length;

a support for holding and maintaining the tissue at its stretched second length; and

30 a sealed container that encloses and maintains the sterility of said submucosal tissue and said support.

24. The package of claim 23, wherein the submucosal tissue has been conditioned to have a strain of less than 30%.

-23-

25. The package of claim 23, wherein the submucosal tissue has been conditioned to have a strain of no more than 20%.

26. The packaged graft material of claim 23
5 wherein the support is a spool, and said submucosal tissue has a first and second end, wherein the first end of the submucosal tissue is fixed onto the spool, the tissue is wrapped onto the spool under tension and the second end is fixed onto the spool to maintain the conditioned state of
10 the submucosal tissue.

27. The packaged graft material of claim 23 wherein the submucosal tissue and the support are sterilized and sealed within a sterilized container.

28. The packaged graft material of claim 23
15 wherein the submucosal tissue comprises the tunica submucosa delaminated from both the tunica muscularis and at least the luminal portions of the tunica mucosa of the intestine of a warm blooded vertebrate species.

1/6

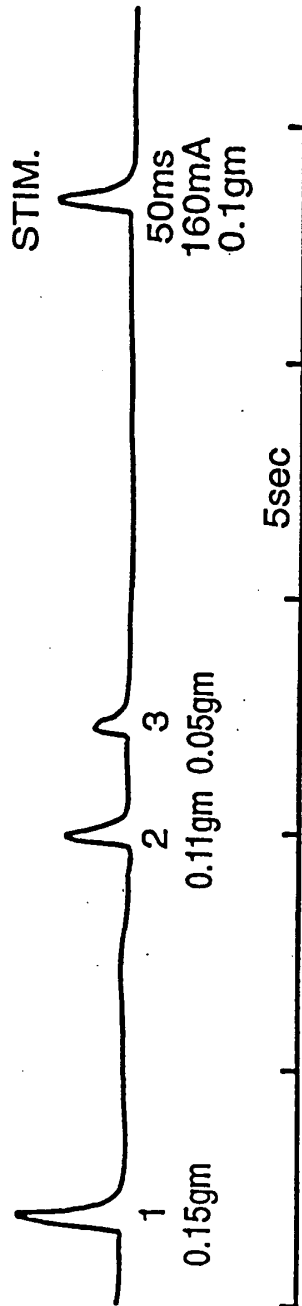


FIG. 1

2 / 6

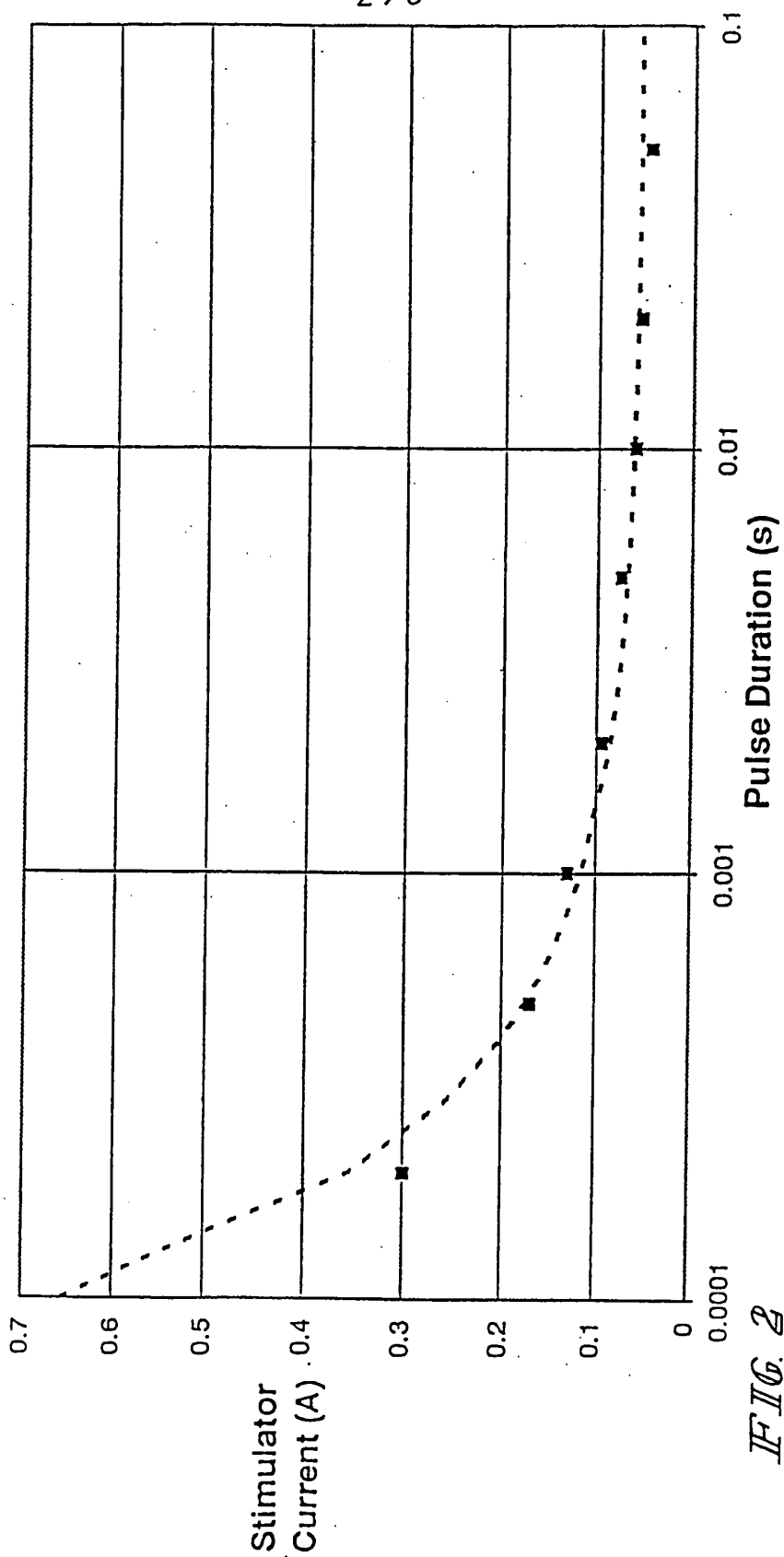


FIG. 2

3/6

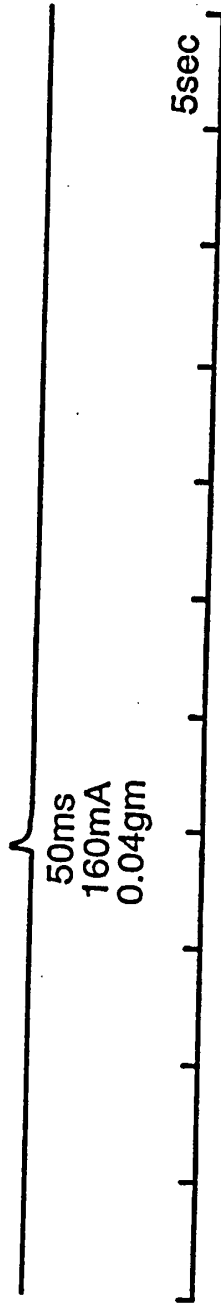


FIG. 3

4 / 6

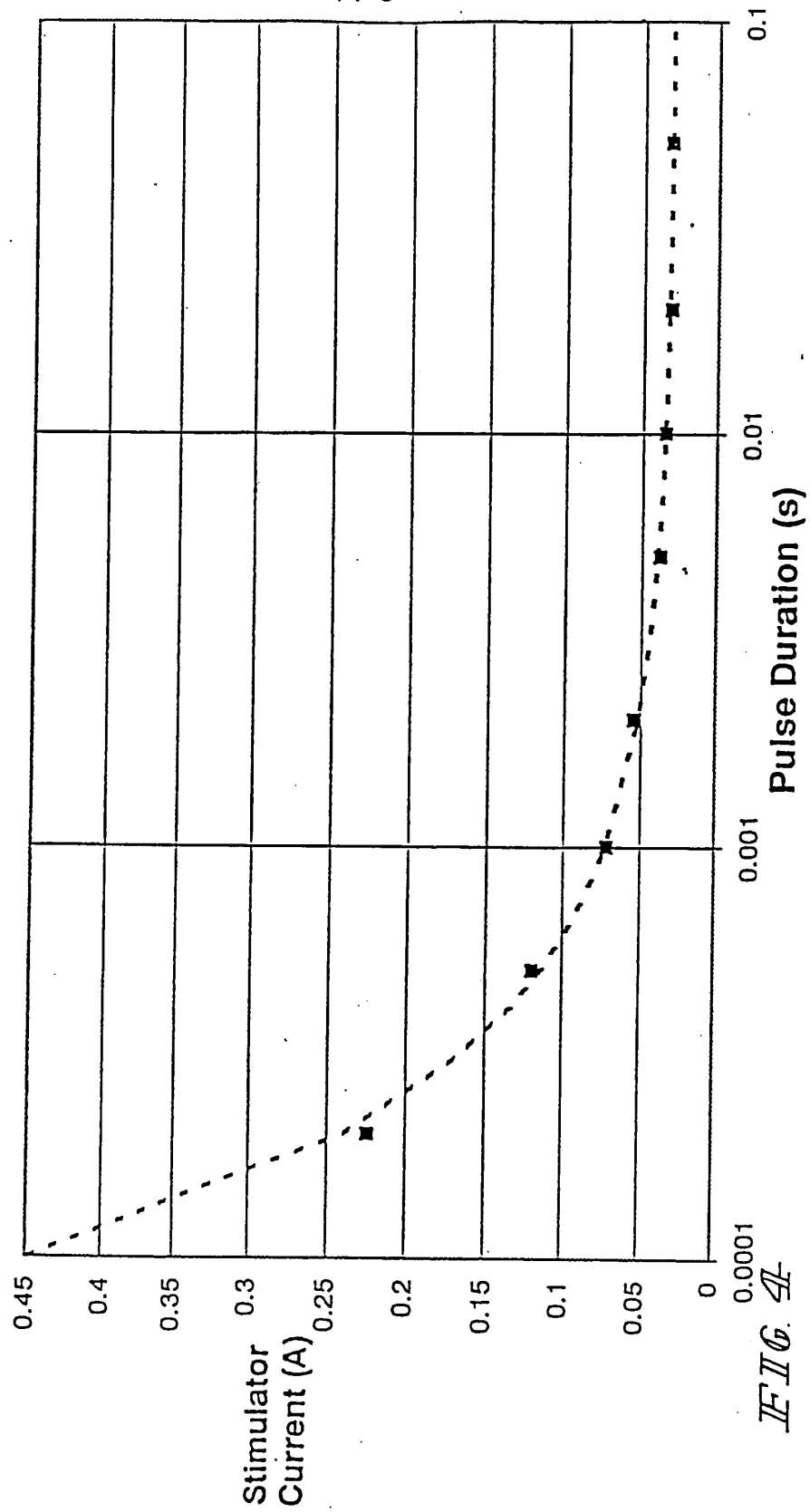
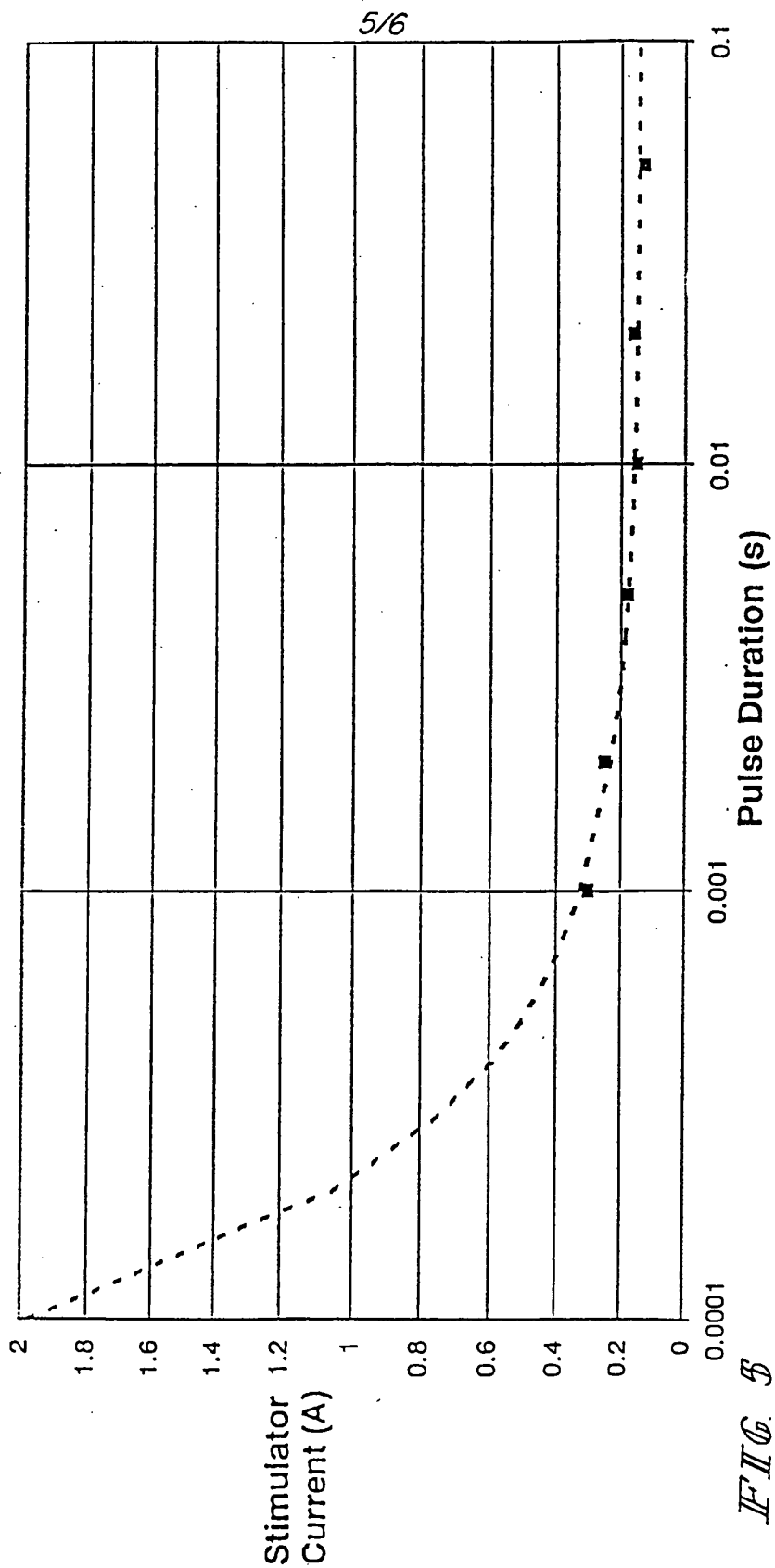


FIG. 4



6/6

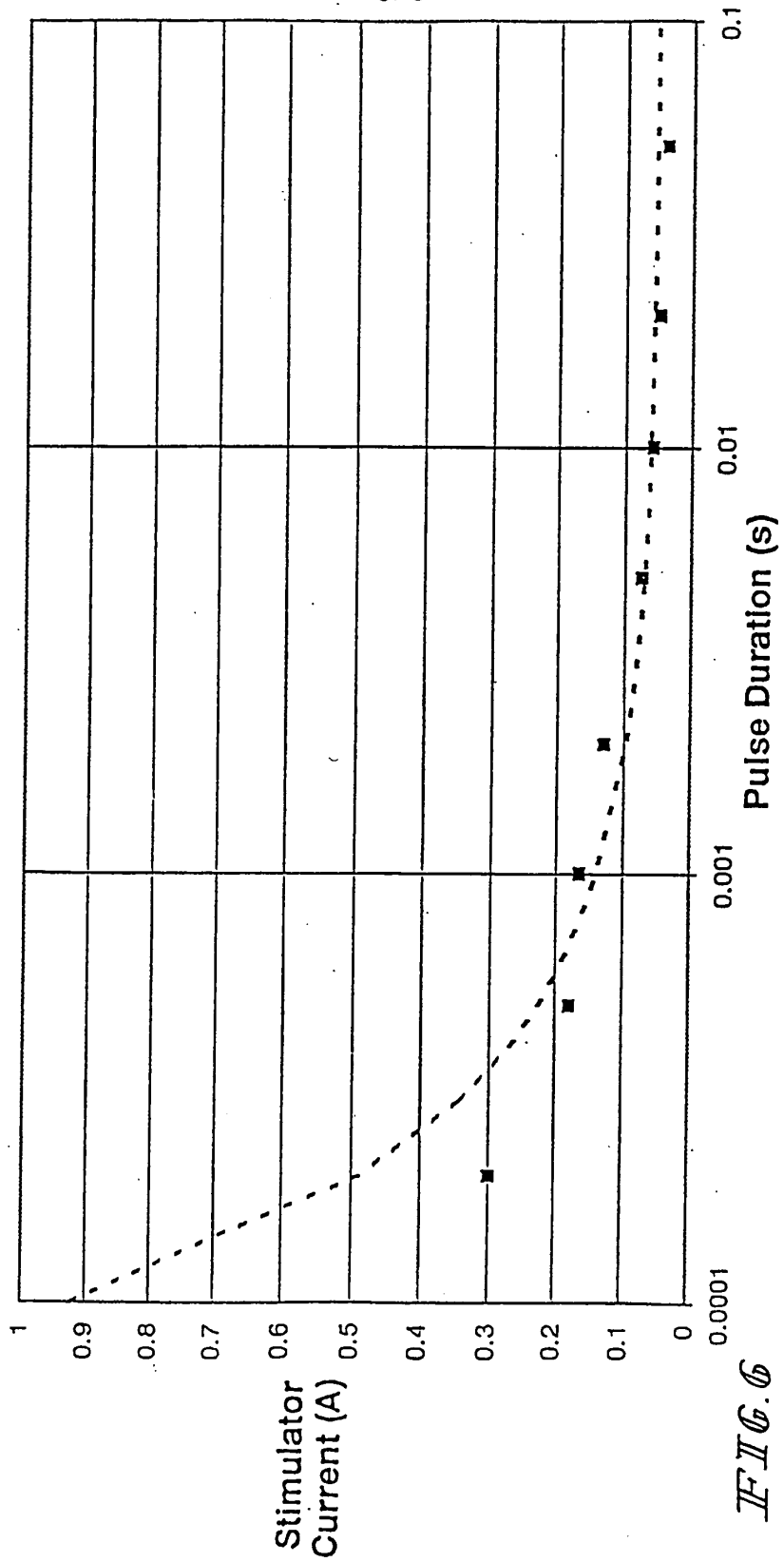


FIG. 6

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/20240

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00 A61K35/38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 31157 A (ORGANOGENESIS INC ;CARR ROBERT M JR (US); TERMIN PAUL L (US); COND) 10 October 1996 see page 12, line 5 - line 8; claims ---	1,5,6,10
X	US 5 480 424 A (COX JAMES L) 2 January 1996 see claims ---	1
P,Y	WO 97 37613 A (DEPUY ORTHOPAEDICS INC ;PLOUHAR PAMELA L (US); DUNN MICHAEL D (US)) 16 October 1997 see claims ---	1-28
Y	WO 96 25179 A (PURDUE RESEARCH FOUNDATION ;BONADIO JEFFREY (US)) 22 August 1996 see claims; examples 1-4 --- -/--	1-28

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

16 March 1998

Date of mailing of the international search report

24.03.98

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

ESPINOSA, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/20240

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 275 826 A (BADYLAK STEPHEN F ET AL) 4 January 1994 cited in the application see the whole document ---	1-28
A	DE 29 29 246 A (INTERMEDICAT GMBH) 7 February 1980 see claims ---	1
A	WO 96 31226 A (PATEL UMESH H ;HILES MICHAEL C (US); WHITSON BRYAN A (US); CHENG B) 10 October 1996 cited in the application see claims; examples ---	1
A	WO 96 31232 A (BADYLAK STEPHEN F ;DEMETER ROBERT J (US); KNAPP PETER M JR (US); L) 10 October 1996 see claims ---	1
A	WO 96 24365 A (PURDUE RESEARCH FOUNDATION) 15 August 1996 see claims ---	1
A	WO 90 00395 A (PURDUE RESEARCH FOUNDATION) 25 January 1990 see claims; examples 1-5 & US 4 902 508 A cited in the application -----	1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/ 20240

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 5 - 16
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/20240

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9631157 A	10-10-96	AU 5308396 A	23-10-96
US 5480424 A	02-01-96	WO 9724082 A	10-07-97
		WO 9724083 A	10-07-97
		US 5713950 A	03-02-98
		AU 4688596 A	28-07-97
WO 9737613 A	16-10-97	NONE	
WO 9625179 A	22-08-96	AU 5296596 A	04-09-96
US 5275826 A	04-01-94	AU 677974 B	15-05-97
		AU 5552394 A	08-06-94
		CA 2149324 A	26-05-94
		CZ 9501240 A	15-11-95
		EP 0671922 A	20-09-95
		FI 952337 A	12-05-95
		HU 73965 A	28-10-96
		JP 8506799 T	23-07-96
		MX 9307046 A	31-01-95
		NO 951881 A	12-05-95
		NZ 258032 A	26-05-97
		PL 309000 A	18-09-95
		SK 61095 A	08-11-95
		WO 9411008 A	26-05-94
		US 5516533 A	14-05-96
		US 5641518 A	24-06-97
DE 2929246 A	07-02-80	NONE	
WO 9631226 A	10-10-96	US 5711969 A	27-01-98
		AU 5536096 A	23-10-96
		CA 2211727 A	10-10-96
		EP 0821590 A	04-02-98
WO 9631232 A	10-10-96	AU 5441396 A	23-10-96
		CA 2214407 A	10-10-96
		EP 0820301 A	28-01-98
		US 5645860 A	08-07-97

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/20240

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9624365 A	15-08-96	US 5641518 A	24-06-97
		AU 4778096 A	27-08-96
		CA 2212647 A	15-08-96
		EP 0808167 A	26-11-97

WO 9000395 A	25-01-90	US 4902508 A	20-02-90
		AT 112963 T	15-11-94
		AU 613499 B	01-08-91
		AU 3709189 A	11-01-90
		CA 1335432 A	02-05-95
		CH 681506 A	15-04-93
		CH 681856 A	15-06-93
		CN 1039352 A,B	07-02-90
		DE 68918943 D	24-11-94
		DE 68918943 T	08-06-95
		DK 340589 A	12-01-90
		EP 0424463 A	02-05-91
		IE 67279 B	20-03-96
		IL 90622 A	24-06-94
		JP 2539934 B	02-10-96
		JP 4501516 T	19-03-92
		MX 171671 B	10-11-93
		OA 9633 A	30-04-93
		PT 91096 B	30-12-94
		RU 2037317 C	19-06-95
		US 4956178 A	11-09-90
